REMARKS

ELECTION / RESTRICTIONS

The Office Action acknowledges Applicants' election without traverse of Group I (claims 107-190) filed February 26, 2004.

Applicants thus acknowledge that claims 107-190 are to be examined. Claims 191-207 have been withdrawn from consideration as being drawn to a non-elected invention, and these claims have been cancelled without prejudice. Applicants reserve the right to pursue any unclaimed subject matter in one or more divisional or continuation applications.

PRIORITY

The Office Action states that claims 107 at step (d), 116 and 170-172 are drawn to a process of direct embryogenesis in monocot plants comprising culturing the primary embryos with auxin, cytokinin, and polyamine at the globular stage to induce organogenesis or until adventitious shoots are noticed, and then regenerating these shoots to plantlets. The Office Action states that these claims are not supported by the specification and claims of the parent application; thus, these claims have an effective filing date equal to the filing of this continuation-in-part application filed August 14, 2001.

Applicants respectfully do not concede to the statements of the Examiner regarding the effective filing date of any portion of any claim. Applicants submit that priority does not need to be addressed for purposes of the present response.

AMENDMENTS TO THE CLAIMS

No new matter has been added in the claims, and entry of these amendments is respectfully requested. Applicant believes that the amended claims define the invention in more explicit and definite terms.

Amended independent claim 107 now recites:

A process for inducing direct somatic embryogenesis in Panicoideae and Pooideae and rapidly regenerating fertile monocotyledonous plants, comprising the steps of:

(a) culturing immature scutella cells of Panicoideae or Pooideae under conditions conducive to direct formation of primary embryos without an intervening callus stage, at least until at least one primary embryo reaches the globular developmental stage;

and one of the following steps selected from:

(b) culturing one or more of the primary embryos from step (a) under conditions conducive to regeneration of plantlets from the primary embryos, and culturing the primary embryo in or on a regeneration medium;

(c) culturing one or more of the primary embryos of Pooideae at the globular developmental stage and no longer than the coleoptilar stage from step (a) under conditions conducive to induction of secondary embryo formation, at least until secondary embryogenesis is detected, and culturing one or more of the secondary embryos under conditions conducive to regeneration of plantlets from the secondary embryos; or

(d) culturing one or more of the primary embryos of Panicoideae at the globular developmental stage and no longer than the coleoptilar stage from step (a) under conditions conducive to induction of organogenesis, at least until adventitious shoots are detected; and culturing the adventitious shoots under conditions conducive to regeneration of plantlets.

For clarity, Applicants have amended claim 107 to refer more precisely to culturing of immature scutella cells of Panicoideae and Pooideae. Support resides in the specification on page 17, lines 11- 19, which define the scutellum, and in Examples 1-7 which describe use of immature scutella culture. For consistency, Applicants have amended claims 108, 124, 125, 127, 130-135, 152,

153, 155, 160-165, 177, 178, 180, and 183-188 to recite "scutella cells." Claims 126, 154 and 179 have thus been withdrawn. Further, several plants within the Poaceae family, which are listed in the specification in the paragraph bridging pages 16 and 17 and in previously presented claims 124, 125, 152, 153 and 177, belong to either the subfamily Panicoideae or Pooideae as follows:

Genera	Subfamily	Support
Triticum	Pooideae	wheat, durum wheat, *Triticum monococum*, *Triticum urartu* used in Examples 2, 3, 7
Hordeum	Pooideae	barley used in Examples 1, 2, 3, 5, 6
Secale	Pooideae	rye used in Example 3
Avena	Pooideae	oat used in Example 3
Dactylis	Pooideae	supported by Examples above
Bromus	Pooideae	supported by Examples above
Lolium	Pooideae	supported by Examples above
Zea	Panicoideae	corn used in Example 4
Sorghum	Panicoideae	sorghum used in Example 4
Pennisetum	Panicoideae	supported by Example 4
Saccharum	Panicoideae	supported by Example 4

Further, Applicants have amended claim 107 to recite that step (c) applies to Pooideae for which support resides in Examples 1-3 and 5-7, while step (d) applies to Panicoideae for which support resides in Example 4.

Before addressing the claim rejections, it is worthwhile to briefly review Applicants' invention, as presently claimed. Monocotyledonous plants, particularly those of the Poaceae family, are known to those skilled in the art to be highly recalcitrant to tissue culture techniques and genetic engineering. In monocotyledonous plants, only a few somatic tissues are capable of being regenerated to form fertile plants. To date, regeneration of fertile monocotyledonous plants

from transformed somatic cells or tissues has remained a challenge, but has now been successfully addressed by Applicants' invention.

Applicants have discovered a surprisingly rapid and efficient species-independent and genotype-independent process for inducing direct somatic embryogenesis and secondary embryogenesis or organogenesis in monocotyledonous plants, and thereafter regenerating fertile and non-chimeric plants. Further, the process is tissue-specific, initiating with the culture of immature scutella cells. Applicants submit that such a process is unique, in that tissue culture protocols, such as those of the cited prior art, are generally very specific with regard to species, genotype, and donor tissue.

Applicants have found that the combination of the two embryogenic stages (i.e., direct somatic embryogenesis and secondary embryogenesis; direct somatic embryogenesis and organogenesis) is surprisingly effective in a wide range of recalcitrant plant species within the Poaceae family, particularly Panicoideae and Pooideae subfamilies. The invention is effective independent of the plant species, as demonstrated in the examples relating to barley genotypes, wheat, durum wheat amphiploids, oat, rye, *Triticum monococum*, *Triticum urartu*, sorghum and corn. Preferred plants include common wheat (*Triticum aestivum*), durum wheat (*Triticum durum*), *Triticum monococum, Triticum urartu,* barley (*Hordeum vulgare*), rye (*Secale cereale*), oat (*Avena sativa*), triticale (*X Triticosecale*), corn (*Zea mays*), sorghum (*Sorghum vulgare*), millet (*Pennesitum glaucum* and *Pennesitum purpureum*), and sugarcane (*Saccharum officinale*), many of which have responded poorly in the past to *in vitro* embryogenesis and regeneration. Applicants emphasize that the invention can be applied to members of the genera *Dactylis* (e.g., orchard grass), *Bromus* (e.g., brome), and *Lolium* (e.g., perennial rye grass), or festuca. Significantly, the processes of the invention are genotype independent, and can be applied, in the case of, for instance, barley or wheat, to all barley and wheat varieties, including malting barley (e.g., cv Harrington), feed barley (e.g., cv AC Lacombe) and forage barley (e.g., T89043003NX), wheat (e.g., cvs AC Nanda and AC Fielder) and durum wheat amphiploids.

Applicants' invention proceeds directly without an intervening callus stage as recited in claim 107. In contrast to prior art tissue culture methods involving indirect somatic embryogenesis, direct somatic embryogenesis avoids a callus step, and its attendant problems, such as increased somaclonal variation and the loss of the ability of cells to regenerate. Few monocot genotypes have been successfully regenerated from calli.

Applicants have discovered that progression of the tissue culture steps on the basis of the developmental stage of the cultured cells as detected by observation, rather than in accordance with a pre-determined time line, is significant. The advantage is that fertile plants regenerate more rapidly than do plants with previous tissue culture methods. Rapid regeneration makes plants less susceptible to developmental abnormalities and provides an opportunity to proceed should the tissue reach the next developmental stage more quickly than anticipated. Claim 107 thus recites that the embryogenic cells are cultured until at least one embryo reaches the globular development stage, and steps (c) and (d) of claim 107 clarify that the primary embryos are cultured at the globular development stage until *de novo* embryo formation (secondary embryos) or *de novo* organogenesis can be detected. The regenerated plants are thus fertile and non-chimeric due to the rapidity of the process.

Briefly, Applicants' invention utilizes immature scutella cells of Poaceae, particularly Panicoideae and Pooideae. The cells are cultured under conditions conducive to direct formation of primary embryos without an intervening callus stage. The cells are cultured in the first step for a period of time sufficient for most of the primary embryos to reach the globular developmental stage.

In a second step, one or more of the globular-stage primary embryos from the first step are cultured under conditions conducive to induction of secondary embryo formation, at least until secondary embryogenesis is detected. The step of secondary embryogenesis circumvents the problem of chimeric embryos by allowing recovery of completely transformed secondary embryos from transformed sectors within a primary somatic embryo. Even if chimeric embryos are still recovered from the first cycle of secondary embryogenesis, continued cycling in the

presence of a selective agent eventually results in embryos consisting entirely of transformed cells. In a third step, the one or more secondary embryos from the second step are cultured under conditions conducive to regeneration of plantlets from the secondary embryos.

Due to variations which naturally occur with somatic embryos and factors such as the species of plant and explant source, cells may have different developmental fates, such that some cells produce embryos (embryogenesis), while others form shoot or root primordia (organogenesis) as discovered by Applicants. In particular species, organogenesis may arise following culturing of globular-stage primary embryos obtained from direct somatic embryogenesis. Applicants have discovered that surprisingly, organogenesis (as detected by at least the formation of adventitious shoots) occurs in Panicoideae subfamilies such as sorghum and corn as demonstrated in Example 4. The adventitious shoots are then cultured to regenerate plantlets. To ensure the successful progression of the embryogenic stages described above, Applicants have further worked out preferred culture media with specific concentrations and relative proportions of plant hormones (auxin, cytokinin and polyamine).

CLAIM REJECTIONS - 35 U.S.C. §112

The Office Action states that in claims 124, 152 and 177, the genus "*Pennesitum*" is misspelled and should be changed to read "*Pennisetum.*"

Applicants have amended claims 124, 152 and 177 to correct the spelling of the word "*Pennisetum.*" Withdrawal of this rejection is thus respectfully requested.

The Office Action states that claims 125 and 153 are indefinite in the recitation of "*amphiploids*" because it is unclear whether "*amphiploids*" applies to Triticum or to all three species. Further, the Office Action states that the word "*amphiploids*" should not be italicized.

Applicants have amended claims 125 and 153 to clarify that reference is made to amphiploids of *Triticum durum* and not to the other species. Withdrawal of this rejection is thus respectfully requested.

CLAIM REJECTIONS - 35 U.S.C. §102

The Office Action rejects claim 107 under 35 U.S.C. 102(a) as being anticipated by Bohanec *et al.* Claim 107 at step (d) is drawn to a method of inducing direct somatic embryogenesis in monocot plant cells comprising culturing primary embryos to induce organogenesis and eventually into plantlets. The Office Action states that Bohanec *et al.* teach a method of inducing direct organogenesis in flower buds of *Allium* in which adventitious shoots develop into plantlets (page 8).

The Office Action further rejects claim 107 under 35 U.S.C. 102(b) as being anticipated by Bohanec *et al.* The Office Action states that claim 107 is drawn to a method of inducing direct somatic embryogenesis in monocot plant cells and then regenerating them into plantlets, and that Bohanec *et al.* teaches a method of inducting direct organogenesis in *Allium* in which adventitious shoots develop into plantlets (page 8).

Further, the Office Action states that claim 107 is rejected as being anticipated by Denchev *et al.* The Office Action states that Denchev *et al.* disclose a method of direct somatic embryogenesis of leaf tissues of orchard grass (page 814) which later develop into plants.

Applicants respectfully traverse this rejection. Since Applicants note that the Office Action has not provided a citation for Bohanec *et al.*, Applicants have relied on International Publication Number WO 00/16610 to Bohanec *et al.* Applicants would appreciate clarification if this is the incorrect reference. Applicants submit that Bohanec *et al.* pertains to a different method involving steps exclusively for use in onion or *Allium cepa* L. (see page 5 of Bohanec *et al.*) Specifically, Bohanec *et al.* relates to a method of direct *in vitro* organogenesis in onion, whereby mature onion flowers or ovaries are induced to form multiple shoot structures. Bohanec *et al.* does not teach use of immature scutella cells, or those of Panicoideae or Pooideae subfamilies, as recited in Applicants' amended claim 107.

Applicants submit that Denchev *et al.* relates to a method for transformation of orchard grass or *Dactylis glomerata* L. involving placement of young leaf tissue (i.e., the basal 30 mm of two innermost leaves) on SH medium supplemented with dicamba, followed by bombardment of

the leaf tissues with DNA coated microprojectiles. Denchev *et al.* focuses predominantly upon enhancing transformation for *gusA* and *bar* genes which is disparate to the subject matter of Applicants' invention. Further, Denchev *et al.* does not teach use of immature scutella cells, or those of Panicoideae or Pooideae subfamilies, as recited in Applicants' amended claim 107. Reconsideration and withdrawal of this rejection of claim 107 is thus respectfully requested.

CLAIM REJECTIONS - 35 U.S.C. §103

The Office Action states that the application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. §103(a), the Examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicants are advised of the obligation under 37 C.F.R. 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the Examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Applicants inform that the subject matter of the various claims has been commonly owned at the time of both initial and later inventions.

The Office Action rejects claims 107-190 under 35 U.S.C. 103(a) as being unpatentable over Denchev *et al.* in view of Mariani *et al.*, Jong *et al.*, Sankhla *et al.*, and Sudhersan *et al.* The Office Action states that Denchev *et al.* disclose leaf tissues of *Dactylis glomerata* (orchard grass) which were genetically transformed by direct somatic embryogenesis from leaf tissues bombarded with DNA coated microprojectiles (page 814). The orchard grass leaf tissues were placed in SH medium with 30 μM of dicamba (page 814, first full paragraph). The Office Action states that Denchev *et al.* noted that the highest ß-glucuronidase expression occurred when the leaf tissues were cultured 48-96 hours before bombardment (page 815 and Figure 1). The Office Action states that transformed embryos grew into plants in which the DNA was extracted from the leaves for analysis. The PCR products were studied by gel electrophoresis and Southern blot

hybridization (pages 814-817). The Office Action states that Denchev *et al.* does not teach the utilization of auxin, cytokinin, and polyamine in the induction, culturing and regeneration media. The Office Action states that Denchev *et al.* does not show that the scutellum is the starting material for direct somatic embryogenesis.

The Office Action states that Mariani *et al.* disclose that direct somatic embryogenesis occurs in rice scutella. Immature caryopsis were dissected and transferred to the induction medium (page 224). After going through the culturing and germinating media eventually grew into plantlets (page 225).

The Office Action states that Jong *et al.* have shown that *Zizania latifolia* (Mancurian wild rice) embryoids were produced from calli grown in B5 medium supplemented with spermine, kinetin and NAA. Jong *et al.* observed that the induced callus taken from the basal segment of the wild rice showed the highest percent of growth (page 68). The Office Action states that these calli develop into embryoids in B5 medium supplemented with 20 mg 1^{-1} spermine, 1 mg 1^{-1} kinetin, and 0.1 1^{-1} NAA (page 69). The embryoids continued to develop into plantlets from the same medium (page 69).

The Office Action states that Sankhla *et al.* disclose that direct somatic embryogenesis occurred without a callus stage in *Echinochloa frumentacea* (Japanese millet). The Office Action states that inflorescence explants were placed on a MS medium supplemented with 5 mg/l 2,4-D and 0.5 mg/l kinetin. Fifteen percent of the cultures exhibit direct somatic embryos (page 369 and Table 2).

The Office Action states that Sudhersan *et al.* teach that high cytokinin induce direct somatic embryogenesis of *Phoenix dactylifera* (date palm). After culturing the leaf tissues in MS medium, Sudhersan *et al.* then transfer the embryogenic calli to 1 mg/l NAA, 3 mg/l 2ip and 3 mg/l kinetin in which plantlets were formed from the higher concentration of cytokinins (page 888).

The Office Action states that it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the culture medium of Denchev *et al.* by

supplementing with auxin, cytokinin and polyamine as taught by Jong *et al.*, and using scutella tissues as the starting material as taught by Mariani *et al.* The Office Action states that claims 124, 125, 152, 153, 177 and 178 are limited to certain species of monocot plants; however, Denchev *et al.* state "any plant system in which a genetically altered cell, by mutation, transformation, etc., can be induced to produce a new genotype through direct shoot or embryo formation" (pages 817-818). The Office Action states that there would have been a reasonable expectation of success given that Sankhla *et al.* has shown that higher cytokinin level will produce organogenesis. The Office Action thus concludes that the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made. Applicants respectfully traverse this rejection.

Applicants have carefully reviewed Denchev *et al.*, and find no suggestion to modify the reference to include Applicants' claim features. Applicants' amended claim 107 relates to use of immature scutella cells of Panicoideae or Pooideae as a starting material. There is no suggestion in Denchev *et al.* to use scutella cells, since Denchev *et al.* relies upon use of the basal 30 mm of two innermost leaves of *Dactylis glomerata* L.

Further, Denchev *et al.* describes use of a single medium (SH medium supplemented with dicamba), and fails to suggest Applicants' preferred culture media with specific concentrations and relative proportions of plant hormones (i.e., auxin, cytokinin and polyamine) which ensure the successful progression of the embryogenic stages as recited in Applicants' claims. Denchev *et al.* focuses predominantly upon bombardment of leaf tissues with DNA coated microprojectiles to enhance transformation for *uidA* and *bar* genes. Denchev *et al.* reports obtaining 16% transgenic embryos and 84% embryos which were either untransformed or chimeric (30%). The very reference relied upon for this 103 rejection did not recognize the advantages to be gained of proceeding with tissue culture steps on the basis of developmental stage of cultured cells as advanced by Applicants. Applicants' rapid regeneration makes plants less susceptible to developmental abnormalities (producing fertile and non-chimeric plants) and

provides an opportunity to proceed as quickly as biologically feasible should the tissue reach the next developmental stage more quickly than anticipated.

Applicants submit that Mariani *et al.* describes surface structural changes during development of rice scutellum as viewed by scanning electron microscopy, and the effects of desiccation treatment on embryo development, which is unrelated to the subject matter of Applicants' invention. Further, Applicants submit that Mariani *et al.* does not teach or suggest progression of the tissue culture steps on the basis of the developmental stage of the cultured cells as recited in Applicants' claim 107. Rather, Mariani *et al.* teaches a process which imposes predetermined time limits on the various tissue culture steps, e.g., 1 week for each step. To Applicants' knowledge, no one, including the cited reference(s), has reported direct somatic embryogenesis in Panicoideae or Pooideae subfamilies. It is well understood by those skilled in the art that tissue culture is dependent upon the tissue type, species and genotype. Applicants submit that the process for direct somatic embryogenesis in rice scutellum as taught in Mariani *et al.* cannot be applied to other species. However, Applicants' invention can be applied successfully to two subfamilies. To Applicants' knowledge, this advance has not been recognized or taught, by any reference, prior to Applicants' present application.

Applicants submit that Jong *et al.* describes induction of callus (derived from young inflorescence explants of *Zizania latifolia* Turcz.) to produce embryoids which later develop into plantlets. In comparison, Applicants' amended claim 107 explicitly recites a process without an intervening callus stage, and use of immature scutella cells of Panicoideae or Pooideae as a starting material. There is no suggestion in Jong *et al.* to use immature scutella cells, since Jong *et al.* refers to a different starting material and different subfamily (i.e., young inflorescence explants of the subfamily Ehrhartoideae).

Applicants submit that Sankhla *et al.* describes culturing of inflorescence explants of *Echinochloa frumentacea* (Japanese millet) to initiate development of three types of callus (see page 368, col. 1, and Figure 1). Somatic embryo formation from each callus type was investigated. Applicants submit that it appears that direct somatic embryogenesis was an artifact

of the embryogenic callus culture process. In contrast, Applicants' amended claim 107 explicitly recites a process without an intervening callus stage. There is no suggestion in Sankhla *et al.* to use immature scutella cells as a starting material. Sankhla *et al.* place inflorescence explants on a MS medium supplemented with 5 mg/l 2,4-D and 0.5 mg/l kinetin. However, to ensure the successful progression of the embryogenic stages, Applicants' invention requires preferred culture media with specific concentrations and relative proportions of three plant hormones (auxin, cytokinin and polyamine). Sankhla *et al.* neither suggests such a combination nor mentions any polyamine.

Applicants submit that Sudhersan *et al.* relates to direct somatic embryogenesis of *Phoenix dactylifera* (date palm) using MS medium containing high cytokinin. There is no suggestion in Sudhersan *et al.* to use immature scutella cells of Panicoideae or Pooideae as recited in Applicants' amended claim 107, since Sudhersan *et al.* uses young leaf lamina of *Phoenix dactylifera* which does not belong to either the Panicoideae or Pooideae subfamilies. Applicants note that Sudhersan *et al.* states that cultures were incubated "for callusing" and "embryogenic calli were transferred to medium" (page 888, second paragraph). Sudhersan *et al.* cultured embryogenic calluses from explant, and then "observed the production of somatic embryos directly on the leaf lamina of the *in vitro* plantlets" (page 888, first paragraph). In contrast, Applicants' claim 107 explicitly relates to a process without an intervening callus stage on the explant. Further, Sudhersan *et al.* fails to teach or suggest Applicants' combination of three plant hormones, particularly inclusion of polyamine.

In that Denchev *et al.*, upon which the 103 rejection is based, is not directed to producing the advantages provided by Applicants' invention, and does not disclose or teach the features of Applicants' claims, there is no motivation to modify the reference teachings whatsoever (with or without the use of other references). It is not seen how any one of the above references, alone or in combination with other references, teaches or renders obvious any of the subject matter of Applicants' claims. Thus it is not believed proper to reject any of these claims, or the claims dependent on these claims, as obvious based on Denchev *et al.* in view of Mariani *et al.*, Jong *et*

al., Sankhla *et al.*, and Sudhersan *et al.* Withdrawal of this rejection of claims 107-190 is respectfully requested.

CONCLUSION

In view of the foregoing, it is submitted that this case is in condition for allowance, and passage to issuance is respectfully requested.

If there are any outstanding issues related to patentability, the courtesy of a telephone interview is requested, and the Examiner is invited to call to arrange a mutually convenient time.

It is believed that this amendment does not necessitate payment of any additional fees under 37 C.F.R. 1.16-1.17. If the amount submitted is incorrect, however, please charge any deficiency or credit any overpayment to Deposit Account No. 07-1969.

Respectfully submitted,

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